Evaluation of the AID AmpC line probe assay for molecular detection of AmpC-producing Enterobacterales

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Abstract: OBJECTIVES In this study, the commercially available AID AmpC line probe assay (LPA) was evaluated for detection of plasmid-mediatedbla$_{AmpC}$ -lactamase genes in Enterobacterales as well as chromosomal mutations in the bla$_{AmpC}$ promoter/attenuator regions in Escherichia coli. METHODS Accuracy of the AID AmpC probes was assessed using Enterobacterales clinical isolates harbouring diverse plasmid-mediated AmpC enzymes (ACC, ACT, DHA, FOX, CMY and MOX) and E. coli clinical isolates with mutations in the chromosomal bla$_{AmpC}$ promoter/attenuator regions. The diagnostic performance of the AID AmpC LPA for bla$_{AmpC}$ detection directly from clinical specimens was determined using 99 clinical urine specimens with bacterial cell counts >10^5CFU/mL and the results were compared with culture-based phenotypic drug susceptibility testing (DST). RESULTS Detection of bla$_{AmpC}$ genes in Enterobacterales clinical isolates showed 100% congruence with phenotypic DST results. The AID AmpC LPA showed 100% specificity [95% confidence interval (CI) 96-100%] and 100% sensitivity (95% CI 75-100%) for detection of plasmid-meditated bla$_{AmpC}$ and E. coli genomic bla$_{AmpC}$ promoter/attenuator mutations directly from clinical urine specimens. The AID AmpC LPA detected three AmpC-producers in urine specimens with bacterial cell counts >10^5CFU/mL that were missed by culture-based phenotypic DST, thereby displaying higher diagnostic sensitivity. CONCLUSION The AID AmpC LPA is an accurate, sensitive and easy-to-use test that can be readily implemented in any diagnostic laboratory for molecular detection of bla$_{AmpC}$ genes in Enterobacterales.

DOI: https://doi.org/10.1016/j.jgar.2019.04.015

Journal of Global Antimicrobial Resistance 19 (2019) 8–13

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

Evaluation of the AID AmpC line probe assay for molecular detection of AmpC-producing Enterobacterales

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ARTICLE INFO

Article history:
Received 31 January 2019
Received in revised form 11 April 2019
Accepted 17 April 2019
Available online 30 April 2019

Keywords:
Molecular detection
AmpC β-lactamase
bla_{AmpC}
Antimicrobial resistance
Enterobacterales

ABSTRACT

Objectives: In this study, the commercially available AID AmpC line probe assay (LPA) was evaluated for detection of plasmid-mediatedbla_{AmpC} β-lactamase genes in Enterobacterales as well as chromosomal mutations in the bla_{AmpC} promoter/attenuator regions in *Escherichia coli*.
Methods: Accuracy of the AID AmpC probes was assessed using Enterobacterales clinical isolates harbouring diverse plasmid-mediated AmpC enzymes (ACC, ACT, DHA, FOX, CMY and MOX) and *E. coli* clinical isolates with mutations in the chromosomal bla_{AmpC} promoter/attenuator regions. The diagnostic performance of the AID AmpC LPA for bla_{AmpC} detection directly from clinical specimens was determined using 99 clinical urine specimens with bacterial cell counts $>10^5$ CFU/mL and the results were compared with culture-based phenotypic drug susceptibility testing (DST).
Results: Detection of bla_{AmpC} genes in Enterobacterales clinical isolates showed 100% congruence with phenotypic DST results. The AID AmpC LPA showed 100% specificity [95% confidence interval (CI) 96–100%] and 100% sensitivity (95% CI 75–100%) for detection of plasmid-meditated bla_{AmpC} and *E. coli* genomic bla_{AmpC} promoter/attenuator mutations directly from clinical urine specimens. The AID AmpC LPA detected three AmpC-producers in urine specimens with bacterial cell counts $>10^5$ CFU/mL that were missed by culture-based phenotypic DST, thereby displaying higher diagnostic sensitivity.
Conclusion: The AID AmpC LPA is an accurate, sensitive and easy-to-use test that can be readily implemented in any diagnostic laboratory for molecular detection of bla_{AmpC} genes in Enterobacterales.

1. Introduction

The prevalence of multidrug-resistant Enterobacterales has increased continuously over the past few years [1] and they are now recognised as a common cause of severe community- and hospital-acquired infections [2,3]. Hence, rapid detection of resistance mechanisms that can compromise standard antibiotic therapies, such as carbapenemases, broad-spectrum β-lactamases, extended-spectrum β-lactamases (ESBLs) and AmpC β-lactamases, in Enterobacterales has become of crucial importance.

In Gram-negative bacteria, AmpC production can be chromosomal or plasmid-mediated. Chromosomal bla_{AmpC} genes are constitutively expressed at low levels. In some Enterobacterales, such as *Citrobacter* spp., *Serratia* spp. and *Enterobacter* spp., chromosomal bla_{AmpC} expression is strongly induced by β-lactam antibiotics. However, mutations in the promoter or attenuator regions of the chromosomal bla_{AmpC} gene may result in constitutive overexpression of AmpC [4,5]. In addition to induction of the bla_{AmpC} gene, Enterobacterales can also acquire plasmid-encoded bla_{AmpC} genes, which are constitutively expressed [6]. The majority of plasmid-mediated bla_{AmpC} genes have been reported in *Escherichia coli* and *Klebsiella pneumoniae* [7–13], although they are also reported in other members of the Enterobacterales [10,14]. AmpC β-lactamases represent a major clinical challenge since they can confer resistance to all β-lactam drugs, except for cefepime, cefpirome and carbapenems [15]. Furthermore, AmpC production in combination with porin defects may also lead to carbapenem resistance [16]. In vitro, tazobactam can inhibit AmpC activity at high drug concentrations. However, a recent clinical study showed non-inferiority of the commonly used piperacillin/tazobactam combination to carbapenems for the treatment of AmpC-producing isolates [17]. Since carbapenem-sparing treatment options can only be implemented with exact microbiological results, precise identification of β-lactamases is thus a genuine medical need.

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https://doi.org/10.1016/j.jgar.2019.04.015

Phenotypic drug susceptibility testing (DST) can detect chromosomal AmpC overexpression and plasmid-mediated AmpC production but is unable to distinguish the different types of plasmid-mediated AmpC β-lactamases [18,19]. Rapid identification of AmpC β-lactamases is necessary for timely implementation of hospital infection control measures and for efficient epidemiological surveillance.

This study evaluated the AID AmpC line probe assay (LPA) (AID Autoimmun Diagnostika GmbH, Straßberg, Germany) for the detection of bla_{AmpC} β-lactamase genes. This assay is designed to identify and differentiate plasmid-mediated bla_{AmpC} genes and point mutations in the chromosomal bla_{AmpC} promoter/attenuator regions responsible for AmpC overexpression in *E. coli*.

2. Methods

2.1. Clinical isolates and DNA fragments used for evaluation of the AID AmpC line probe assay

A collection of 168 non-duplicate clinical Enterobacterales isolates was used to determine the diagnostic performance of the AID AmpC LPA. All 168 isolates had been previously phenotypically analysed for the presence of AmpC enzymes [20]. Of the 168 clinical Enterobacterales isolates, 38 (22.6%) were found to be AmpC β-lactamase-producers by phenotypic DST [20], and the results were confirmed by multiplex PCR [21].

For evaluation of the diagnostic performance of the LPA probes, DNA fragments corresponding to plasmid-mediated bla_{AmpC} [bla_{FOX} (1149 bp, GenBank accession no. **NG_049098.1**), bla_{MOX} (1149 bp, accession no. **NG_049311.1**), bla_{ACC} (1161 bp, accession no. **NG_048588.1**) and bla_{ACT-1} (1147 bp, accession no. **NG_048597.1**)] as well as *E. coli* chromosomal bla_{AmpC} sequences with mutations in the promoter (bimut, −15 ln t, −15 ln tg) and attenuator regions (mut2, del2) that were not available from clinical Enterobacterales isolates were synthesised and cloned into the pUC57-plasmid by Genscript Biotech. Corp. (Piscataway, NJ, USA).

2.2. Culture and phenotypic determination of AmpC production

Retrospective analysis of antibiograms from bacteria isolated from urine specimens (sent from secondary- or tertiary-care hospitals) in the routine bacteriology laboratory of the Institute of Medical Microbiology of the University of Zurich (Zurich, Switzerland) was performed to determine the prevalence of AmpC-producers in the Zurich metropolitan area (Supplementary Fig. S1).

Clinical specimens were analysed for the presence of AmpC β-lactamase-producing isolates using culture and phenotypic DST. Briefly, 10 μL of urine specimen was placed onto Columbia agar with 5% sheep blood (bioMérieux SA, Marcy-l'Étoile, France), chromogenic UriSelect™4 agar (Bio-Rad Laboratories, Hercules, CA, USA) and Columbia colistin/nalidixic acid agar with 5% sheep blood (bioMérieux SA) and was incubated on average for 16 h. Species-level identification of bacteria was performed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) (Brucker, Bremen, Germany). Antimicrobial susceptibility to cephalosporins was determined by the disk diffusion method using 30 μg cefoxitin disks (i2a, Montpellier, France) on Mueller–Hinton agar plates (Becton Dickinson, Allschwil, Switzerland) employing the interpretative criteria recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [22]. When the cefoxitin inhibition zone diameter was <19 mm, phenotypic DST for cefoxitin was repeated in combination with cloxacillin. Enterobacterales were considered AmpC-producers when a ≥4 mm increase in the inhibition zone diameter was observed compared with cefoxitin alone [20].

2.3. AID AmpC line probe assay testing

The AID AmpC LPA can detect plasmid-mediated bla_{AmpC} genes (bla_{ACC}, bla_{ACT}, bla_{DHA}, bla_{FOX}, bla_{CMY} and bla_{MOX}) as well as mutations in the *E. coli* bla_{AmpC} promoter (−42, −32, bimut, −18/−1, −15 ln g, −15 ln t, −15 ln tg) and attenuator regions (mut, mut2, del1, del2) (Supplementary Fig. S2). Testing of the AID AmpC LPA (AID Autoimmun Diagnostika GmbH) was performed following the manufacturer's instructions. Briefly, DNA was extracted from bacterial isolates using InstaGene Matrix (Bio-Rad, Reinach, Switzerland). DNA was extracted from 200 μL of urine specimen on a QIAsymphony system (QIAGEN, Hilden, Germany) with the QIA DSP Virus/Pathogen Kit. PCR amplification was performed with primers specific for the bla_{AmpC} target genes, and PCR amplicons were hybridised to the probes immobilised on the LPA.

The AID AmpC LPA was assessed as interpretable if it showed (i) a positive hybridisation signal with the conjugate and amplification control, (ii) a clear hybridisation signal with the promoter/attenuator wild-type or mutant probes or (iii) a clear hybridisation signal with one of the plasmidic bla_{AmpC} probes. The AID AmpC LPA was assessed as uninterpretable if it showed (i) no hybridisation signal with the conjugate and amplification control or (ii) a weak hybridisation signal with the bla_{AmpC} wild-type probes.

2.4. AID AmpC line probe assay testing from clinical urine specimens

To evaluate the diagnostic performance of the AID AmpC LPA directly from clinical specimens, first the limit of detection of the AID AmpC LPA from urine specimens was determined (Supplementary Methods; Supplementary Fig. S3). Next, a prospective study was performed using a set of 99 urine samples with bacterial cell counts $>10^5$ CFU/mL from unique patients with urinary tract infection (UTI) collected from May–October 2017 at secondary- or tertiary-care hospitals in the Zurich metropolitan area. These 99 urine specimens were analysed in parallel by AID AmpC LPA and phenotypic DST.

2.5. Discrepancy analysis

A 2 × 2 contingency table was used to display the results from the AID AmpC LPA and culture-based phenotypic DST for the 99 urine specimens. To resolve discrepant results between phenotypic DST and AID AmpC LPA in three urine specimens, a 271-bp fragment of the *E. coli* bla_{AmpC} promoter/attenuator region was amplified using primers AB1 (5′-GATCGTTCTGCCGCTGTG-3′) and ampC2 (5′-GGGCAGCAAATGTGGAGCAA-3′) [23]. Plasmid-mediated bla_{AmpC} genes were amplified by PCR using the primers previously described by Pérez-Pérez and Hanson [21]. Sequences of PCR products were then verified by Sanger sequencing. Finally, this compiled gold standard (i.e. culture-based phenotypic DST and Sanger sequencing) was used for evaluation of the diagnostic performance of the AID AmpC LPA directly from urine specimens.

2.6. Ethics

The research study was conducted in accordance with the Declaration of Helsinki and national and institutional standards. The Act on medical research involving human subjects does not apply to this study. This study was approved by the Ethical Committee of the Canton of Zurich, Switzerland.

3. Results

3.1. Diagnostic performance of the AID AmpC line probe assay

The accuracy of the AID AmpC probes was assessed using clinical *E. coli* isolates with chromosomal bla_{AmpC} promoter (–18/–1mut, –15 ln g, –32mut and –42mut) and attenuator mutations (mut and del2) as well as *E. coli* isolates harbouring bla_{DHA} and bla_{CMY} genes. In addition, *E. coli* isolates carrying synthetic gene constructs with plasmidic bla_{AmpC} genes (bla_{ACC}, bla_{ACT}, bla_{FOX} and bla_{MOX}) or chromosomal bla_{AmpC} promoter (bimut, –15 ln t, –15 ln g, –15 ln tg) and attenuator mutations (mut2 and del1) were used for evaluation of the AID AmpC LPA. The AID AmpC LPA detected the corresponding bla_{AmpC} genes with 100% accuracy (Table 1).

The diagnostic performance of the AID AmpC LPA was investigated using 168 selected clinical Enterobacterales isolates (116 *E. coli*, 33 *K. pneumoniae*, 14 *Klebsiella oxytoca* and 5 *Citrobacter koseri*; Table 2) including 38 AmpC-producers and 130 non-AmpC-producers. Overall, the AID AmpC LPA exhibited excellent performance, showing complete congruence with phenotypic DST results (Table 2).

3.2. Phenotypic versus genotypic detection of AmpC-producing Enterobacterales directly from clinical urine specimens

The limit of detection of the AID AmpC LPA directly from urine specimens was a bacterial cell count of $>10^5$ CFU/mL (Supplementary Fig. S3), corresponding to the widely accepted threshold for a relevant bacteriuria. Subsequently, 99 urine specimens above the limit of detection were subjected to the AID AmpC LPA and phenotypic DST. Of the 99 urine specimens, 3 (3.0%) showed a weak hybridisation signals with the *E. coli* bla_{AmpC} promoter/attenuator wild-type probes on the AID AmpC LPA that could not be interpreted as either 'positive' or 'negative'. From these three urine specimens, cultured *E. coli* isolates showed no AmpC production as determined by phenotypic DST, which is consistent with the hybridisation signals on the LPA (i.e. *E. coli* bla_{AmpC} promoter/attenuator wild-type; Table 3). Nevertheless, as hybridisation signals on the LPA could not be initially interpreted, these three LPAs were scored as uninterpretable and the urine samples were subsequently excluded from the study.

In ten Enterobacterales that were phenotypically resistant to cefoxitin (inhibition zone diameter <19 mm), the AID AmpC LPA detected plasmid-mediated bla_{AmpC} genes in nine isolates and bla_{AmpC} promoter mutations in one *E. coli* isolate (Tables 3 and 4). Of 96 Enterobacterales isolates tested, 83 (86.5%) were phenotypically susceptible to cefoxitin and all tested negative by the AID AmpC LPA. In three urine specimens, AmpC-producers were identified by the AID AmpC LPA but the cultured *E. coli* isolates were phenotypically susceptible to cefoxitin (inhibition zone diameter ≥19 mm) (Table 3). PCR amplification and sequencing directly from the cultured isolates confirmed the presence of a plasmid-mediated DHA bla_{AmpC} gene in one isolate and a chromosomal bla_{AmpC} promoter mutation (mut –18/–1) in the other two *E. coli* isolates (Table 4). This resulted in 100% specificity [95% confidence interval (CI) 96–100%] and 100% sensitivity (95% CI 75–100%) for bla_{AmpC} detection directly from urine specimens by the AID AmpC LPA.

4. Discussion

This study investigated the diagnostic performance of the new, commercially available AID AmpC LPA targeting plasmid-mediated bla_{AmpC} genes (bla_{ACC}, bla_{ACT}, bla_{DHA}, bla_{FOX}, bla_{CMY} and bla_{MOX}) as well as mutations in the chromosomal *E. coli* bla_{AmpC} promoter/attenuator regions. To our knowledge, the AID AmpC LPA is

Table 1
Analysis of AmpC-producing Enterobacterales isolates or synthetic bla_{AmpC} genes using the AID AmpC line probe assay.

Bacterial isolate/synthetic DNA	Plasmid-mediated bla_{AmpC} [a]						Chromosomal bla_{AmpC} [a,b]													
	ACC	ACT	DHA	FOX	CMY	MOX	Prom wt	Prom –42mut	Prom –32mut	Prom bimut	Prom –2 wt	Prom –18/–1mut	Prom –15 ln g	Prom –15 ln t	Prom –15 ln tg	Atten wt	Atten mut	Atten mut2	Atten del1	Atten del2
Plasmid-mediated bla_{AmpC}																				
ACC syn	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
ACT syn	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
DHA *E. coli*	–	–	+	–	–	–	+	–	–	–	+	–	–	–	–	+	–	–	–	–
FOX syn	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
CMY *E. coli*	–	–	–	–	+	–	+	–	–	–	+	–	–	–	–	+	–	–	–	–
MOX syn	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Chromosomal *E. coli* promoter/attenuator																				
Prom wt *E. coli*	–	–	–	–	–	–	+	–	–	–	+	–	–	–	–	+	–	–	–	–
Prom –42mut *E. coli*	–	–	–	–	–	–	–	+	–	–	+	–	–	–	–	+	–	–	–	–
Prom –32mut *E. coli*	–	–	–	–	–	–	–	–	+	–	+	–	–	–	–	+	–	–	–	–
Prom bimut syn	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–	+	–	–	–	–
Prom –2 wt *E. coli*	–	–	–	–	–	–	+	–	–	–	+	–	–	–	–	+	–	–	–	–
Prom –18/–1mut *E.coli*	–	–	–	–	–	–	+	–	–	–	–	+	–	–	–	+	–	–	–	–
Prom –15 ln g *E. coli*	–	–	–	–	–	–	+	–	–	–	–	–	+	–	–	+	–	–	–	–
Prom –15 ln t syn	–	–	–	–	–	–	+	–	–	–	–	–	–	+	–	+	–	–	–	–
Prom –15 ln tg syn	–	–	–	–	–	–	+	–	–	–	–	–	–	–	+	+	–	–	–	–
Atten mut *E. coli*	–	–	–	–	–	–	+	–	–	–	+	–	–	–	–	–	+	–	–	–
Atten mut2 syn	–	–	–	–	–	–	+	–	–	–	+	–	–	–	–	–	–	+	–	–
Atten del1 *E. coli*	–	–	–	–	–	–	+	–	–	–	+	–	–	–	–	–	–	–	+	–
Atten del2 syn	–	–	–	–	–	–	+	–	–	–	+	–	–	–	–	–	–	–	–	+

Atten, attenuator; prom, promoter; syn, synthetic DNA; wt, wild-type.
[a]The genotype of all *Escherichia coli* isolates has been independently verified by multiplex PCR or sequencing.
[b]Wild-type (wt) probes highlighted in grey.

Table 2
Analysis of 168 clinical Enterobacterales isolates using the AID AmpC line probe assay.

Bacterial isolate	Interpretation		Plasmid-mediated *bla*$_{AmpC}$ [a]						*Escherichia coli* chromosomal AmpC promoter/attenuator [a,b]												
	wt	AmpC-producer	ACC	ACT	DHA	FOX	CMY	MOX	Prom wt	Prom –42mut	Prom –32mut	Prom bimut	Prom –2 wt	Prom –18/–1mut	Prom –15 ln g	Prom –15 ln t	Prom –15 ln tg	Atten wt	Atten mut	Atten mut2	Atten del1	Atten del2
E. coli	79								79				79					79				
E. coli		3							3					3				3				
E. coli		3									3		3					3				
E. coli		2									2			2				2				
E. coli		2								2				2				2				
E. coli		1							1						1			1				
E. coli		6							6				6						6			
E. coli		3							3				3									3
E. coli		11					11		11				11					11				
E. coli		2					2		2					2				2				
E. coli		2					2		2				2						2			
E. coli		1			1				1				1					1				
E. coli		1			1				1					1				1				
K. pneumoniae	32																					
K. pneumoniae		1					1															
K. oxytoca	14																					
C. koseri	5																					
Total	130	38 [c,d]			2		16		109	2	5		105	10	1			105	8			3

Atten, attenuator; ESBL, extended-spectrum β-lactamase; prom, promoter; wt, wild-type.
[a]AmpC production has been independently verified in all strains by phenotypic drug susceptibility testing, PCR amplification and sequencing.
[b]Wild-type (wt) probes displayed in grey.
[c]Nine AmpC-producing *E. coli* isolates additionally produced an ESBL; one isolate additionally produced an ESBL and a carbapenemase.
[d]The AmpC-producing *Klebsiella pneumoniae* isolate additionally produced an ESBL and a carbapenemase.

Table 3
Characterisation of AmpC-producing Enterobacterales in clinical urine specimens ($n = 99$ with bacterial cell count $>10^5$ CFU/mL) by culture-based phenotypic drug susceptibility testing (DST) and the AID AmpC line probe assay (LPA).

AID AmpC LPA	Culture and phenotypic DST, PCR amplification and sequencing	
	No AmpC-producers detected	AmpC-producers detected
No *bla*$_{AmpC}$ detected	86[a]	0
bla$_{AmpC}$ detected	0	13[b]

[a] Three AID AmpC LPAs showed weak hybridisation signals with the *Escherichia coli bla*$_{AmpC}$ promoter/attenuator wild-type probes and were determined as non-AmpC-producers by culture-based phenotypic DST.
[b] A plasmid-mediated *bla*$_{AmpC}$ gene was identified in one *E. coli* isolate, and an *E. coli bla*$_{AmpC}$ promoter mutation (promoter −18/−1mut) was confirmed in two *E. coli* isolates by PCR amplification and Sanger sequencing.

Table 4
Detection of *bla*$_{AmpC}$ genes directly from clinical urine specimens ($n = 13$) by the AID AmpC line probe assay (LPA).

Culture and phenotypic DST			AID AmpC LPA result[a]
Species identification	Cell count (CFU/mL)	FOX inhibition zone diameter (<19 mm)	
Klebsiella pneumoniae	$>10^5$	+	DHA
Citrobacter freundii	$>10^5$	+	CMY
C. freundii	$>10^5$	+	CMY
Hafnia alvei	$>10^5$	+	ACC
Enterobacter cloacae complex	$>10^5$	+	ACT
Morganella morganii	$>10^5$	+	DHA
Escherichia coli	$>10^5$	+	–42 and –18/–1 promoter mut
K. pneumoniae	$>10^5$	+	ACT
Klebsiella oxytoca	$>10^5$	+	ACT
C. freundii	$>10^5$	+	CMY
E. coli	$>10^5$	FOX-susceptible	DHA
E. coli	$>10^5$	FOX-susceptible	−18/−1 promoter mut
E. coli	$>10^5$	FOX-susceptible	−18/−1 promoter mut

DST, drug susceptibility testing; FOX, cefoxitin.
[a] The AID AmpC LPA results have been independently verified by PCR amplification and Sanger sequencing.

currently the only commercially available assay enabling broad molecular identification of bla_{AmpC} genes in Enterobacterales.

Overall, the AID AmpC LPA exhibited excellent diagnostic performance with clinical Enterobacterales isolates. All bla_{AmpC} genes were correctly identified in phenotypic AmpC-producers, and all non-AmpC-producers were correctly identified, resulting in 100% congruence with phenotypic DST results. The concomitant presence of ESBLs or carbapenemases in some Enterobacterales isolates did not interfere with bla_{AmpC} detection by the AID AmpC LPA. This represents a competitive advantage over culture-based phenotypic DST that is often unable to identify multiple β-lactamases on the basis of minimum inhibitory concentration (MIC) patterns [24,25].

Moreover, the AID AmpC LPA can be used for rapid detection of AmpC-producers directly from clinical urine specimens (with bacterial cell counts $>10^5$ CFU/mL). The AID AmpC LPA is faster and easier to perform than culture-based phenotypic DST and provided accurate molecular detection and differentiation of bla_{AmpC} genes (chromosomal versus plasmid-mediated AmpC). Furthermore, it was more sensitive than culture-based detection of AmpC β-lactamases. However, reliable AmpC detection by the AID AmpC LPA required bacterial cell counts $>10^5$ CFU/mL. Thus, pre-screening of urine specimens is advisable in order to reduce unnecessary molecular testing. To this end, flow cytometers such as the UF-4000 (Sysmex Europe GmbH, Norderstedt, Germany) or other bacterial cell counting techniques that enable rapid determination of bacterial cell numbers in urine specimens could be employed prior to AID AmpC LPA molecular testing.

Several cases of nosocomial outbreaks caused by Enterobacterales with plasmid-encoded AmpC β-lactamases have been reported in recent years [9,26,27]. In a prospective surveillance study conducted in Switzerland in 2012, the prevalence of AmpC-producers was found to be 0.16% in Enterobacterales and 0.2% in *E. coli* [28]. In the current study, a marked increase in AmpC-producers was observed among Enterobacterales causing UTIs (4%). This observation is alarming given the strict policy of antibiotic prescription in Switzerland [29]. Enterobacterales producing plasmid-mediated AmpC β-lactamases raise special concern because of the high rate of clinical failure among patients treated with standard antibiotic regimens [30–32]. Moreover, plasmid-encoded bla_{AmpC} genes in Enterobacterales are often associated with additional resistance determinants [33–35], further limiting therapeutic options. AmpC-producers can be detected by culture-based phenotypic DST. Costs for urine culture and phenotypic DST are low (less than €12 per bacterial isolate) but they have a turnaround time of ≥2 days. In contrast, implementation of molecular methods enables more rapid identification (<1 working day) and precise characterisation of AmpC β-lactamases. Molecular assays for detection of plasmid-mediated bla_{AmpC} genes, such as the multiplex PCR developed by Pérez-Pérez and Hanson [21], are now widely used in diagnostic practice. Moreover, a PCR reverse hybridisation assay for detection of ACT, ACC-1, DHA, MOX, FOX and CMY-2-type β-lactamase genes has been recently developed [36]. However, these assays cannot detect mutations in the *E. coli* bla_{AmpC} promoter/attenuator regions, requiring time-consuming PCR amplification and DNA sequencing. Moreover, commercial assays offer some advantages compared with molecular tests developed 'in-house'. First, manufacturing of the assays is strictly controlled and standardised to ensure test accuracy and reproducibility. Chemicals and kit reagents have a specific shelf-life and a lot number for diagnostic laboratory use, and laboratory protocols have been extensively validated ensuring consistency in the performance of the assay. Also, whole-genome sequencing (WGS) has been successfully employed for the detection of genes and/or mutations that result in phenotypic drug resistance in Enterobacterales [37]. However, WGS is still costly and slow (more than €100 per bacterial isolate; turnaround time of 2–3 working days) and requires expensive equipment (sequencers, library preparation kits, etc.) and highly trained staff (bioinformaticians, etc.). In contrast, LPAs are cheap (less than €25), fast (<1 working day), easy to use and can readily be employed in every diagnostic laboratory with a PCR machine available, enabling molecular bla_{AmpC} detection also in resource-limited settings.

The most significant limitation of this study is the low prevalence of some AmpC types (e.g. ACC, ACT, FOX and MOX) in our region. Therefore, the reliability in detecting these rarely occurring bla_{AmpC} genes by the AID AmpC LPA needs to be assessed by sequencing of the respective bla_{AmpC} genes. Another limitation of this study is that the majority of bla_{AmpC} genes were detected in *E. coli*. Although bla_{AmpC} genes in four *Klebsiella* spp. (from one culture isolate and three urine specimens) and three *Citrobacter freundii* (from urine specimens) were correctly identified, and 45 *Klebsiella* spp. and 7 *Citrobacter* spp. without plasmidic bla_{AmpC} genes did not show hybridisation signals on the AID AmpC LPA, analysis of a greater number of non-*E. coli* strains carrying bla_{AmpC} genes is required to corroborate the analytical performances of this method.

In conclusion, the AID AmpC LPA is a rapid and accurate molecular assay that allows precise characterisation of plasmid-mediated and chromosomal bla_{AmpC} genes in Enterobacterales. It can easily be implemented in diagnostic laboratories and be used for early detection of antimicrobial resistance, to guide antibiotic therapy and to monitor resistance epidemiology.

Funding

This study was funded by the Institute of Medical Microbiology of the University of Zurich.

Competing interests

ECB is a consultant of AID Autoimmun Diagnostika GmbH. All other authors declare no competing interests.

Ethical approval

The research study was conducted in accordance with the Declaration of Helsinki and national and institutional standards. The study was approved by the Ethical Committee of the Canton of Zurich, Switzerland [Req-2018-00472].

Acknowledgments

The authors thank Prof. Reinhard Zbinden, Valéria P. Pires and the technicians of the Institute of Medical Microbiology (IMM) of the University of Zurich (Zurich, Switzerland) for expert help and assistance. The authors also thank the IMM for continuous support.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jgar.2019.04.015.

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